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REPORT NUMBER 806

BIOCHEMICAL PROFILES OF SUBMARINERS:
A LONGITUDINAL HEALTH STUDY

by

Donald V. Tappan, Michael J. Jacey, Elly Heyder,
and William A. Tansey

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MR041.06.01-0026.02

Released by:

R. L. Sphar, CDR MC USN
COMMANDING OFFICER
Naval Submarine Medical Research Laboratory

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SUMMARY PAGE

THE PROBLEM

To obtain biochemical profile data for evaluation of the initial health status of submariners participating in a longitudinal health study and to establish criteria for comparisons with future data.

FINDINGS

Essentially normal data were recorded for 13 biochemical parameters included in the study. The frequency distributions of the data vary from Gaussian normality to markedly skewed. Such distributions, however, seem to follow patterns observed in similar normal population groups.

APPLICATION

The present findings are essential to a comprehensive study of the longitudinal health effects of submarine service, and they will constitute the basis for future comparisons. The distribution patterns of the biochemical data presented should prove especially helpful in providing interpretive insight into biomedical changes that may occur in this population.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Work Unit MR041.06.01-0026 - "Environmental Stress. The present report is Number 2 on this work unit. It was submitted for review on 10 January 1975, approved for publication on 15 May 1975 and designated as NavSubMedRschLab Report Number 806.

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ABSTRACT

As part of the Naval Submarine Medical Research Laboratory's continuing Longitudinal Health Study, the sera of 566 submariners were analyzed for 12 biochemical parameters, using standardized automated procedures. In addition, a two-hour glucose tolerance test was performed on each participant. Mean values of all parameters measured were within generally accepted normal clinical limits. The values for cholesterol, uric acid, inorganic phosphate, and glucose were shown to be distributed according to Gaussian probability curves. A slight skewing was demonstrated for total calcium, lactic dehydrogenase, alkaline phosphatase, and total protein. Blood urea nitrogen, serum glutamic-oxalacetic transaminase, albumin, and bilirubin exhibited marked skewness. The significance of these distributions is discussed.

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BIOCHEMICAL PROFILES OF SUBMARINERS: A LONGITUDINAL HEALTH STUDY

INTRODUCTION

The Submarine Service is a powerful deterrent against international conflict. Nuclear-powered submarines operating from unknown locations afford our nation an effective system of security. The Submarine Service, its men and ships, is and will continue to be, a vital factor in maintaining the integrity of this nation and the free world.

The preservation of the health and well-being of the crews that operate this important defense system is a major consideration of Naval biomedical research. The closed submarine environment and its related physical and emotional stresses have been studied and described by various investigators^{6,12,16,22,23}.

The Longitudinal Health Study of the Naval Submarine Medical Research Laboratory is a continuing multiphasic medical survey of officers and enlisted men serving in submarine activities^{17,19}. The purpose of this study is to define the health characteristics of the members of this population, describe changes in their medical status, and ultimately improve health, safety and well-being of this select group of Naval personnel.

The phase of data acquisition reported here commenced in April 1973 and lasted until January 1974. During this period, 566 submariners were processed through the multiphasic health survey. It is the intent of this report to describe the biochemical profiles of these subjects as representative members of the "Silent Service".

METHODS AND MATERIALS

The subjects, all qualified submariners, were recruited from various activities afloat as well as ashore. They were requested to ingest nothing after 2400 on the night prior to reporting for the Longitudinal Health Study examination.

Upon reporting for study at 0700, a 7.5-ml vacuum tube without anticoagulant was used to obtain blood without stasis from the antecubital vein. Immediately thereafter, 100 gms of glucose were administered orally to each subject for a glucose-tolerance test. Two hours later, another sample of blood was drawn in the same manner from each participant.

The two tubes of blood were allowed to clot adequately, were centrifuged, and the sera removed. An aliquot of serum from each tube was analyzed for its glucose content utilizing the Diagnostest system (Dow Chemical Co.).

A second aliquot of fasting serum was used to determine the standard 12 biochemical parameters measured by the Technicon SMA 12/60 system according to the methodology existing in 1973¹⁵. The remaining sera were then frozen and stored in a frozen serum bank.

Frequency distributions were constructed for the 12 standard biochemical parameters, glucose-tolerance data, and age. These distribution curves were then utilized to calculate skewness according to the Pearsonian model³.

RESULTS

The duty status and age information of the subjects are given in Table 1. The frequency curve for age is depicted in Figure 1. As indicated in the table, approximately 90% of the participants in these studies were on active duty aboard nuclear submarines. The average age of the subjects was 28.3 years with a range of 18-42.

TABLE 1
DUTY STATUS AND AGE INFORMATION FOR
PARTICIPANTS IN THIS STUDY

Number of subjects	SSBN 439	SSN 71	SHORE 56
% OF TOTAL	77.5%	12.5%	10%
AGE			
Mean		28.29	
SEM		.241	
N		566	

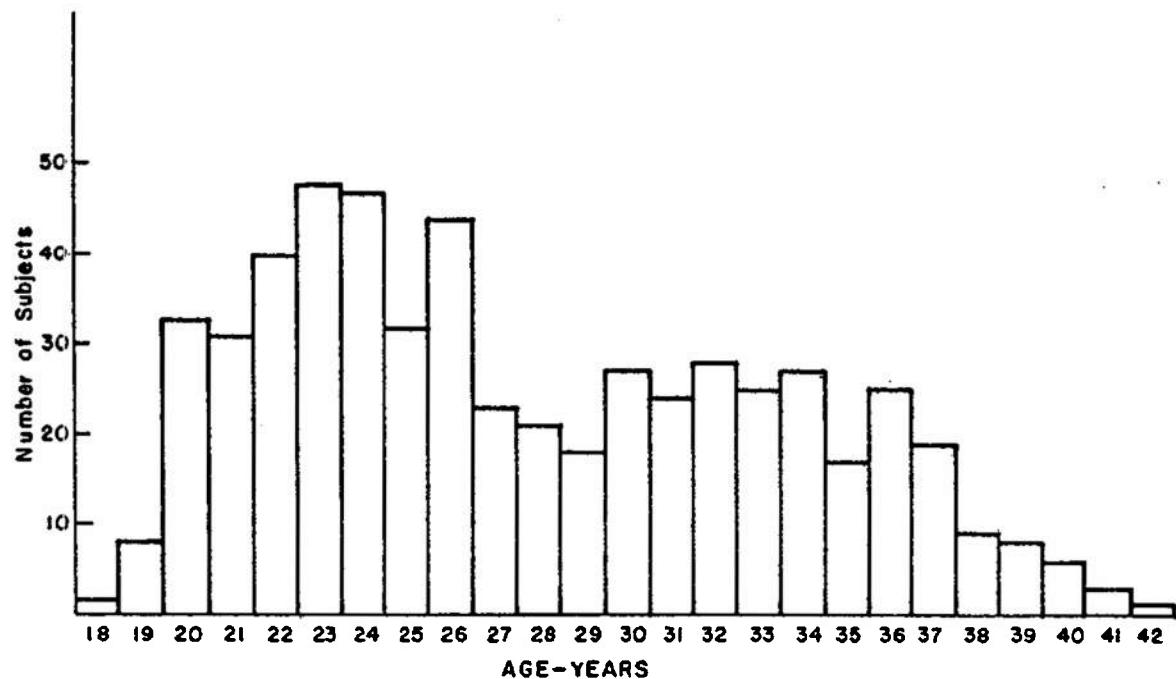


Fig. 1. Frequency distribution of ages of participants in the study

A statistical analysis of the SMA 12/60 test results of 566 submariners is presented in Table 2. The mean values

reported indicate an essentially normal biochemical profile for the group.

TABLE 2

STANDARD BIOCHEMICAL PROFILES AS DETERMINED BY A TECHNICON SMA 12/60
N = 566

	Calcium mg/dl	Inorganic Phosphate mg/dl	Glucose mg/dl	Blood Urea Nitrogen mg/dl	Uric Acid mg/dl	Cholesterol mg/dl
Mean	9.74	4.10	103.50	16.27	6.41	208.96
S.E.M.	.019	.021	.592	.141	.041	1.467
Total Protein gm/dl	Albumin gm/dl	Total Bilirubin mg/dl	Alkaline Phosphatase mU/ml	Lactic Dehydrogenase mU/ml	Serum Glutamic-Oxalacetic Transaminase mU/ml	
Mean	7.43	4.41	.70	69.60	171.67	44.26
S.E.M.	.017	.031	.017	.776	1.56	.68

Frequency distributions of these 12 standard biochemical parameters are shown as follows: Figure 2--Calcium (Total), Inorganic Phosphorus, Glucose,

and Blood Urea Nitrogen; Figure 3--Uric acid, Cholesterol, Total Protein, and Albumin; Figure 4--Total Bilirubin, Alkaline Phosphatase, Lactic Dehydrogenase, and Serum Glutamic-Oxalacetic Transaminase.

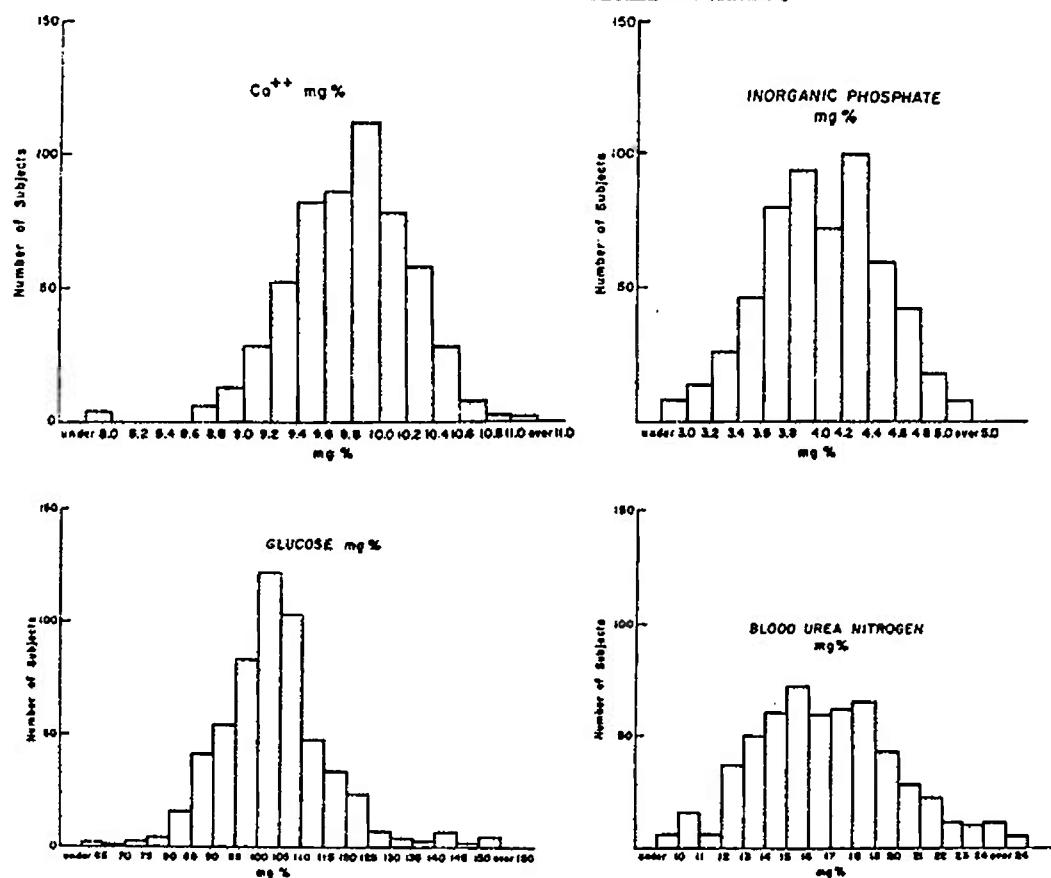


Fig. 2. Frequency distributions for (a) Calcium (Total), (b) Inorganic Phosphate, (c) Glucose, and (d) Blood Urea Nitrogen data measured in sera of submarine personnel

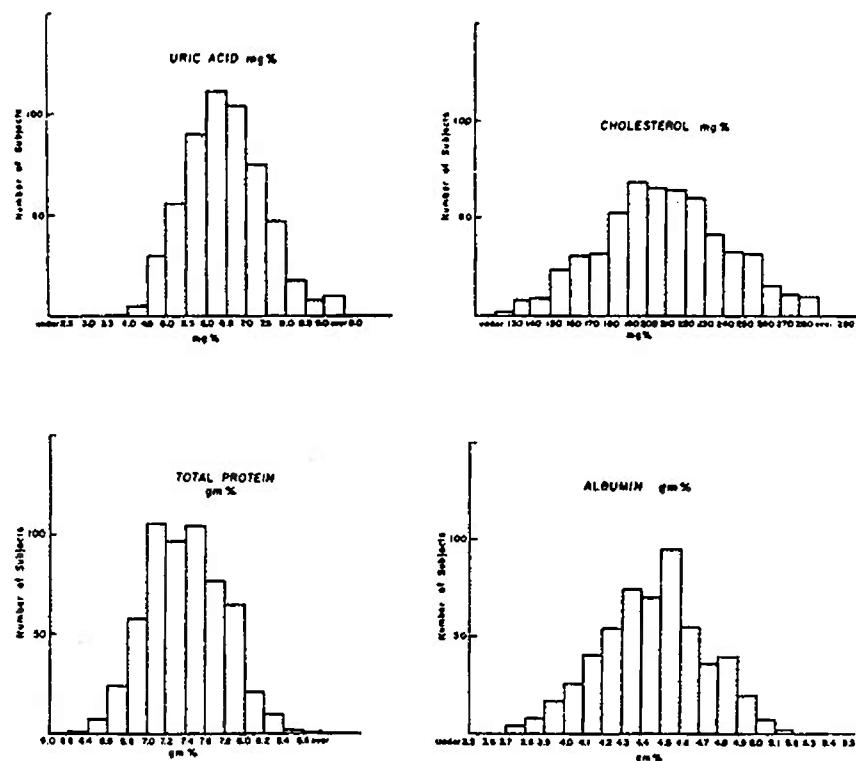


Fig. 3. Frequency distribution for (a) Uric Acid, (b) Cholesterol, (c) Total Protein, and (d) Albumin data measured in sera of submarine personnel

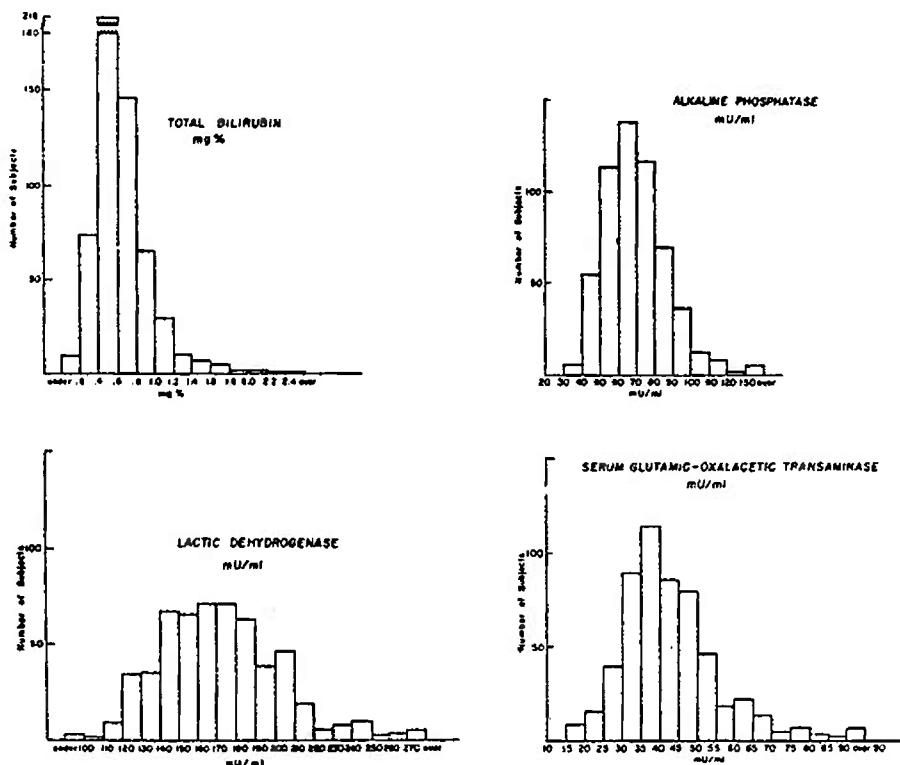


Fig. 4. Frequency distribution for (a) Total Bilirubin, (b) Alkaline Phosphatase, (c) Lactic Dehydrogenase, and (d) Serum Glutamic-Oxalacetic Transaminase measured in sera of submarine personnel

The results of the two hour glucose tolerance tests and their frequency distributions are shown in Table 3 and Figure 5, respectively. For the sake of easy evaluation of the biochemical profile of this population of submarine personnel, the average value for each para-

meter has been drawn on a SMA 12/60 printout sheet as if the 566 subjects were a single individual. The printout is illustrated in Figure 6 with median and skewness of the frequency distribution of the SMA 12/60 data and the two-hour glucose-tolerance results given in Table 4.

TABLE 3

GLUCOSE TOLERANCE DATA FROM
A SUBMARINE POPULATION

	Fasting Glucose (mg/dl)	Glucose 2 hours after oral administration of 100 gm glucose (mg/dl)
Mean	88.03	91.84
SEM	.615	.963
N	566	553

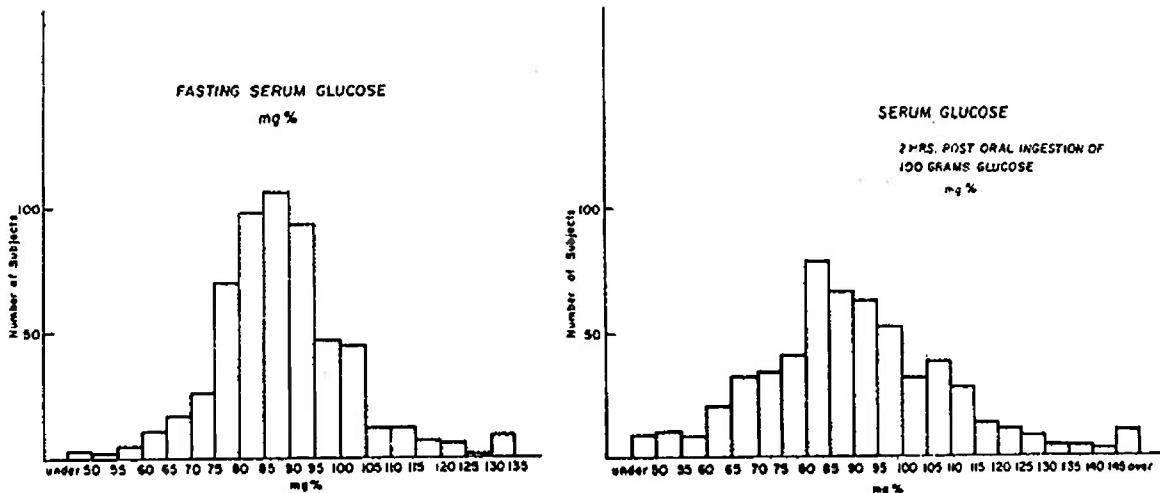


Fig. 5. Frequency distribution of results of two-hour glucose tolerance tests performed on submarine personnel

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TWELVE-SIXTY

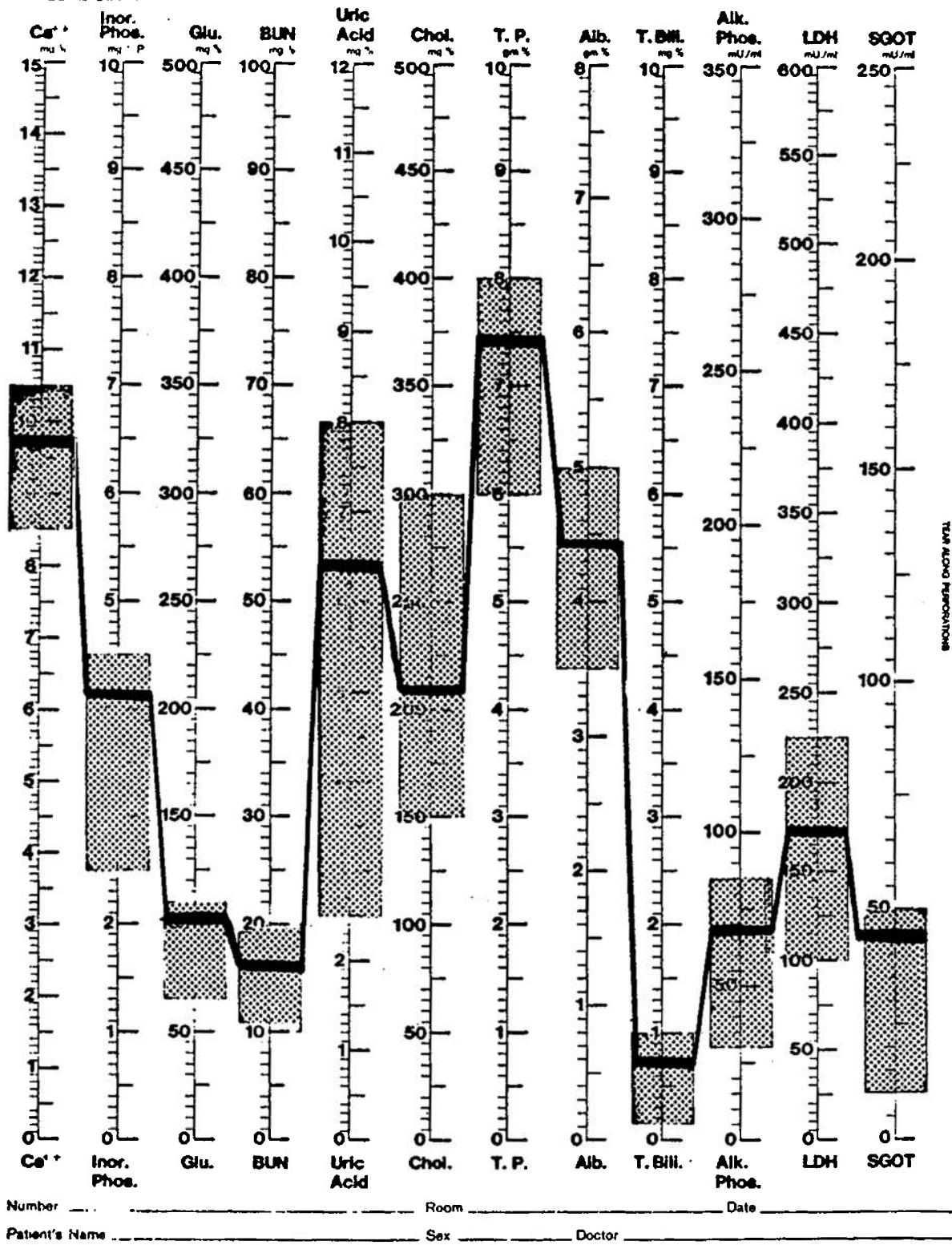


Fig. 6. Composite SMA printout sheet for all 12 biochemical parameters

TABLE 4

Median and Skewness of the Frequency Distributions of the Standard SMA Parameters and Glucose Data as Determined on 566 Submariners

Parameters	Median Value of the Distribution*	Skewness of the Distribution
1. Cholesterol	208.33	.02
2. Uric Acid	6.42	-.03
3. Inorganic Phosphate	4.5	-.03
4. Glucose	103.17	.07
5. Calcium	9.72	.13
6. Lactic Dehydrogenase	169.01	.22
7. Alkaline Phosphatase	67.90	.28
8. Total Protein	7.38	.38
9. Blood Urea Nitrogen	15.58	.64
10. Serum Glutamic - Oxalacetic Transaminase	40.46	.65
11. Albumin	4.47	-.69
12. Bilirubin	.58	.90
<hr/>		
1 - Fasting Glucose	87.36 mg/dl	.14
2 - Glucose - 2 Hours Post Glucose Load (100 gm)	88.78 mg/dl	.46

* See Table 2 for units

It should be noted that while a few qualified divers are included in this study, all subjects were serving in

submarine activities at the time of this examination.

DISCUSSION

A longitudinal health study, by definition, requires comparison of several increments of data obtained on the same population over a period of years. Since this study details the information obtained during the first phase of screening, it is not possible to compare these results to any previous data. By necessity then, the only meaningful comparisons that can be made at this time are those using previously published material on multiphasic screening studies involving large groups of presumably healthy males ages 20-40. As will be discussed presently, the mean values for all 12 standard biochemical parameters, as well as the glucose-tolerance test data, fall within generally accepted ranges of normality. The 95% confidence range of normality, or the range covering two standard deviations from the mean, is shown as the gray shaded area on the strip-chart recording output of the SMA 12/60.^{2,5}

Each parameter measured will be discussed in turn as it relates to the total submarine environment. All comparisons made to the findings of other multiphasic studies in this portion of the discussion specifically refer to healthy males, 20-40 years old.

Calcium (total): This mineral has been studied in the sera of submariners by Heyder⁹. No changes were reported as a result of prolonged exposure to the submarine environment. The average calcium values obtained in the present study compares well with those of other investigations^{2,4,13}.

Inorganic Phosphorus: This mineral was also measured by Heyder⁹ in sera of submarine crews. She found no changes related to the submarine sojourn. Other workers have also reported results similar to ours in studies on young healthy males^{4,13}.

Glucose: The mean values for fasting blood sugar reported by Cutler and associates⁴ and Craig and Bartholomew² are essentially identical to our average values of 103.5 mg/dl.

Blood Urea Nitrogen: Tolls et al,²⁰ and Craig and Bartholomew² found serum concentrations of this protein metabolite similar to those reported in the current study.

Uric Acid: Our mean value also agrees with those measured by other investigators^{2,4,13} for similar age groups. This metabolic end product was assayed by Campbell and Rahe¹ during exposure to the total submarine atmosphere. No significant changes were noted.

Cholesterol: The mean cholesterol level determined here is approximately the same as that seen in a similar American male population^{4,20}. This steroidal compound has also been studied by Shrivtaker¹⁸ in sera of crew members during a Polaris submarine patrol. He found that cholesterol levels increased during the patrol and concluded that emotional stress and the lack of exercise, coupled with a diet rich in saturated fats, were major factors in development of the hypercholesterolemia. Moreover, Campbell and Rahe¹ detected a slight tendency for cholesterol values to increase toward the end of a patrol. These authors suggested that this change is due to the possibility that the patrols become slightly less enjoyable near their termination.

Total Protein: Results comparable to ours were obtained by Werner and co-workers²¹.

Albumin: Werner and co-workers²¹ also obtained values for this protein similar to those reported in our study.

Bilirubin: This breakdown product of senescent erythrocytes was assayed in a population study by Tolls and associates²⁰. Concentrations similar to those measured by us were found in sera.

Alkaline Phosphatase: The activity of this ubiquitous enzyme was also quantitated by Tolls et al.²⁰ who demonstrated activity levels similar to those reported here.

Lactic Dehydrogenase: This glycolytic enzyme has been shown by Jacey and Schaefer¹¹ to exhibit circadian periodicity. The nadir of activity occurs at approximately 0800 with blood samples drawn during P.M. hours yielding higher readings. Although all sera for SMA 12/60 analysis were drawn at 0700, the mean enzyme activity in sera of submariners was 20 mU/ml higher than that reported by Tolls et al.²⁰. The accepted range of lactic dehydrogenase activity, however, is sufficiently broad that the mean value reported here is well within normal limits¹³.

Serum Glutamic-Oxalacetic Transaminase: The activity of this enzyme of protein metabolism was found to average 44.26 mU/ml in sera of submariners. According to our survey of previously published multiphasic biomedical studies, normal means for large populations of young males were usually lower. However, in 8015 healthy subjects, O'Rell and Elliott¹³ found apparently abnormal results for 13.1%, which suggests the possibility that normal values may vary widely. On the other hand, Reid¹⁶ has measured the activity of this enzyme in sera of a crew aboard a Polaris submarine during deployment and detected no increase in activity as a result of exposure to the total submarine environment.

Glucose-Tolerance Test: The results of this test document normal carbohydrate metabolism for the group; the two-hour postload glucose value being almost equal to the fasting values. The apparent discrepancy of 14mg/dl between the SMA 12/60 analysis and the ortho-toluidine procedures employed for the glucose-tolerance studies is due to methodology; 90 mg/dl being the accepted mean for the ortho-toluidine technique¹⁰.

To estimate the degree of skewness in the frequency distributions of the biochemical parameters studied, the Pearsonian analysis was employed³. If no skewing is present, the resulting expression has a value of 0; its maximum value approaches ± 3 as limits. A negative value indicates skewing to the left while a positive value denotes skewing to the right.

On the basis of the calculated skewness values, the distribution curves of the 12 standard biochemical parameters may be grouped into three separate categories: Gaussian--cholesterol, uric acid, inorganic phosphate and glucose; slightly skewed--total calcium, lactic dehydrogenase, alkaline phosphatase, and total protein; strongly skewed--blood urea nitrogen, serum glutamic-oxalacetic transaminase and bilirubin. The two-hour glucose-tolerance test results also demonstrate a definite skewing.

A statistical approach utilized by Dinio and fellow workers⁵ to study frequency distributions in 2,500 SMA 12/60 test results from healthy subjects demonstrated that some distributions do not fit a Gaussian probability

curve. It is the contention of these authors that for populations which are distributed in a Gaussian manner, the central 90% are included in the definitely healthy part of the group. On the other hand, when Gaussian distribution is lacking only the central 80% of the population may be considered to be normal. Since the men included in the present study were carefully screened before becoming submariners and represent a very select, healthy population, we conclude that skewed frequency distributions may indeed occur normally for many biochemical parameters.

The atmosphere of a nuclear submarine is replete with a wide variety of environmental contaminants, organic as well as inorganic^{12,16}. They exhibit wide fluctuations from ship to ship and from time to time within the same ship¹². The principal atmospheric contaminant continues to be CO₂ (.7-.9%). While a review of the pertinent literature^{7,8,9,14} indicates that no serious pathological consequences have been proved for CO₂ or other contaminants, many of the contaminating materials require extensive additional study to adequately evaluate their long-term biological significance.

For future investigators who may study biochemical profiles of submariners, a note of caution is appropriate. The results generated for this first group of subjects have been obtained by methodology in use as of mid-1973¹⁵. A new generation of automated analyzers with different analytical procedures and different normal ranges is already on the scene. Meaningful comparisons in the future can only be made when the differences in techniques and methods are taken into consideration.

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REFERENCES

1. Campbell, D. T. and R. T. Rahe. Serum uric acid and cholesterol variability for men aboard a Polaris submarine. Milit Med 141: 462-465, 1974.
2. Craig, J. L. and M.D. Bartholomew. Blood profile ranges by age decades in 7,337 male employees. In: Advances in Automated Analysis-Technicon International Congress 1969. Vol. III. Biomedical Profiling. Mediad Inc., White Plains, New York, 1970, pp. 105-109.
3. Croxton, F. E. Elementary statistics with applications in medicine and the biological sciences. Dover Publications, New York, New York, 1959
4. Cutler, J. L., M. F. Collen, A. B. Siegelaub, and R. Fieldman. Normal values for multiphasic screening blood chemistry tests. In: Advances in Automated Analysis - Technicon International Congress 1969, Vol III. Biomedical Profiling. Mediad Inc., White Plains, New York, 1970, pp. 67-73.
5. Dino, R. C., G. Raminex, and H. C. Pribor. Pattern recognition of SMA 12 values as a diagnostic tool. In: Advances in Automated Analysis-Technicon International Congress 1969, Vol I. Clinical Analysis. Thurman Associates, Miami, Florida, 1971, pp. 201-209.
6. Earls, J. H. Human adjustment to an exotic environment. The Nuclear Submarine. Arch Gen Psychiatry 20: 117-123, 1969.
7. Gortner, D. A., A. A. Messier, E. Heyder, and K. E. Schaefer. The effects of elevated atmospheric CO₂ on acid-base balance and red-cell electrolytes of FBM submarine crew members. NAVSUBMEDRSCHLAB Report #692, 1973.
8. Hester, R. Provisional medical statistics for personnel attached to nuclear powered submarines FY 66-69. NAVSUBMEDRSCHLAB Report #674, 1971.
9. Heyder, E. Studies of calcium and inorganic phosphorus levels in plasma and erythrocytes during acute and chronic hypercapnia. NAVSUBMEDRSCHLAB Report #702, 1974.
10. Indriksons, A. The Dow Chemical Co., Indianapolis, Ind. Personal Communication. August 1974.
11. Jacey, M. J. and K. E. Schaefer. Circadian cycles of lactic dehydrogenase in urine and blood plasma: Response to high pressure. Aerospace Med 39: 410-412, 1968.
12. Musick, J. K. and F. S. Thomas. Determining the suitability of oxidation catalysts for purifying the atmospheres of nuclear submarines.

- NAVRSCHLAB Report #752,
1973.
13. O'Rell, R. T. and J. R. Elliot. Development of normal values for use in multitest biochemical screening of sera. Clin Chem 16: 161-165, 1970.
14. Peck, A. S., Jr. The time course of acid-base balance while on FBM submarine patrol. NAVSUBMEDRSCHLAB Report #675, 1971.
15. Reagent data handbook for the Technicon SMA 12/60 System. Technical Publication NO. THO-0160-11, Revised January 1973. Technicon Instruments Corp., Tarrytown, New York.
16. Reid, J. W. A study of liver function on Polaris patrol. Unpublished thesis - School of Submarine Medicine, at U. S. Naval Submarine Base New London, Groton, Connecticut, 1966.
17. Sawyer, R. N. with J. H. Baker. The Longitudinal health survey: I. Description. NAVSUBMEDRSCHLAB Report #733, 1972.
18. Shrivtaker, L. W. Cholesterol levels in submariners: A pilot study. NAVSUBMEDRSCHLAB Report #771, 1974.
19. Tansey, W. A. The longitudinal health study: A multiphasic medical surveillance program for U.S. Navy submarine and diving personnel. NAVSUBMEDRSCHLAB Report #786, 1974.
20. Tolls, R. E., M. Werner, J. V. Hultin, and J. Mellecker. Sex and age dependence of seven serum constituents in a large ambulatory population. In: Advances in Automated Analysis - Technicon International Congress 1969, Vol. III. Biomedical Profiling. Mediad Inc., White Plains, New York, 1970, pp. 9-14.
21. Werner, M., R. E. Tolls, J. V. Hultin, and J. Mellecker. Sex and age dependence of serum calcium, inorganic phosphorus, total protein, and albumin in a large ambulatory population. In: Advances in Automated Analysis - Technicon International Congress 1969, Vol. III. Biomedical Profiling. Mediad Inc., White Plains, New York, 1970, pp. 59-65.
22. Weybrew, B. B. Submarine crew effectiveness during submerged missions of sixty days duration. NAVSUBMEDRSCHLAB Report #686, 1971.
23. Wilken, D. A. Significant medical experiences aboard Polaris submarines. NAVSUBMEDRSCHLAB Report #560, 1969.

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13. ABSTRACT
As part of the Naval Submarine Medical Research Laboratory's Longitudinal Health Study, the sera of 566 submariners were analyzed for 12 biochemical parameters using standardized automated procedures. In addition, a two-hour glucose tolerance test was performed on each participant. Mean values of all parameters measured were within generally accepted normal clinical limits. The values for cholesterol, uric acid, inorganic phosphate, and glucose were shown to be distributed according to Gaussian probability curves. A slight skewing was demonstrated for total calcium, lactic dehydrogenase, alkaline phosphatase, and total protein. Blood urea nitrogen, serum glutamic-oxalacetic transaminase, albumin, and bilirubin exhibited marked skewness. The significance of these distributions is discussed.

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